

Enzyme Studies May Lead to New Antiviral Agents

Funded by DOE and the National Institutes of Health, three new enzyme studies at BNL have yielded a new strategy for blocking infection by human adenovirus. The findings, reported in the October, November, and December, 2001 issues of the journal, *Biochemistry*, have already been used to design novel antiviral drugs.

Adenoviruses cause a number of acute infections, including respiratory and gastrointestinal infections, and conjunctivitis. In patients with compromised immune systems, such as those infected with human immunodeficiency virus (HIV), an opportunistic adenovirus infection, is frequently deadly.

"Our new antiviral drugs are expected not only to inhibit adenovirus, but also, possibly, to be effective against other organisms that use the same enzyme - including *Chlamydia*, one of the most prevalent sexually transmitted bacteria, and *Yersinia pestis*, the organism that causes the black plague," said Walter Mangel, Biology Department, the lead scientist on the studies.

Infection Process

During infection, these viruses make an enzyme called a protease, which cleaves or degrades other proteins. The protease is used by the virus to complete the maturation of newly synthesized virus particles.

To explain this process, Mangel uses the example of building a cathedral around internal scaffolding. Once the cathedral is in place, the last step is to remove the scaffolding. "Similarly," says Mangel, "adenovirus particles are built with scaffolding proteins inside. Once

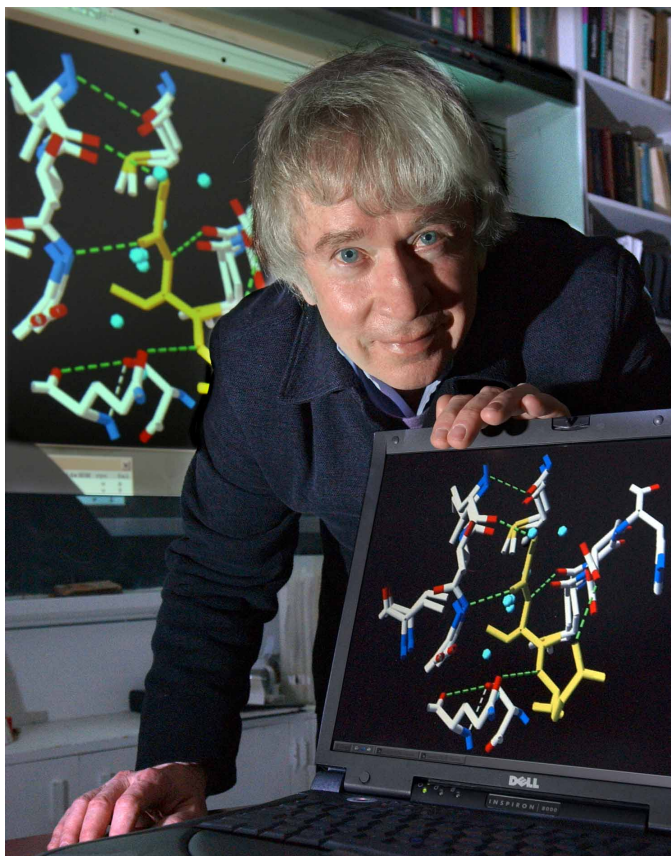
the virus particle is formed, the protease becomes activated and cleaves the scaffolding to render the virus particle infectious."

The three recent studies at BNL reveal that the protease is initially synthesized in an inactive form. The inactive enzyme binds to the viral DNA to become partially activated.

The partially activated enzyme then cleaves out a cofactor, which is a protein fragment that binds to the protease to activate it further. The fully active complex of enzyme and cofactor then moves along the viral DNA, cleaving the scaffolding proteins.

"Such activation of a protease by DNA has never been seen before," Mangel said. And this presented a problem.

"Two other laboratories repeatedly claimed in the literature that they saw no stimulation of the enzyme by DNA," Mangel continued. "So, in one of the three papers, we not only presented definitive evidence that the enzyme interacted with DNA, but also showed why the two other groups had not seen stimulation of the enzyme by DNA. Essentially, they did not use the correct conditions. We hope that, with the publication of these papers, this controversy is now resolved."



Walter Mangel

Next Step: To Design Drugs

"These studies suggest that drugs that bind to the active site of the enzyme, which is the part involved in cleaving proteins, the cofactor binding site, or the DNA binding site should block the enzyme's action and serve as effective antiviral agents," Mangel said.

To design drugs able to bind to and block these sites, the scientists first had to characterize the molecular structures. The active site of the enzyme had been previously characterized by William McGrath, a postdoc in Mangel's team, using an intense beam of x-rays available at the NSLS. The pattern of x-rays bouncing off the atoms reveals the three-dimensional molecular structure.

In the current studies, Stony Brook University graduate student Mary Lynn Baniecki characterized the binding of the cofactor to the pro-

tease, identifying which parts bind to the enzyme and which parts stimulate the enzyme's activity. McGrath and Baniecki then deciphered how the protease binds to the DNA.

Among the other coauthors of the study, Mangel said, are three undergraduate students, David Green, Caroline Li, and Sarah McWhirter, who were participants in the DOE Energy Research Undergraduate Laboratory Fellowships program managed at BNL by the Office of Educational Programs.

New, Three-Pronged Therapy

Based on the findings, Mangel has proposed a new form of antiviral therapy using three different drugs against these three target sites - the active site, the cofactor binding site, and the DNA binding site - on the same virus-coded protein. This three-pronged approach may overcome one of the biggest chal-

lenges in antiviral therapy - the spontaneous evolution of drug-resistant strains.

As Mangel explains the idea, a mutation conferring drug resistance at one site may alter the physiological functions at the other two sites because the three sites are interdependent, thereby making drug resistance much less likely to arise.

"The adenovirus protease may be a good model system within which to test the efficacy of this form of combination therapy," Mangel said. Already, his team has developed two new drugs, one that binds reversibly and another irreversibly to the active site of the protease. These drugs will soon be tested as antiviral agents by the National Institutes of Health.

- Karen McNulty Walsh

[Editor's note: Reprinted with permission from the BNL Bulletin - January 18, 2002.]



A graphic representation of the structure of the adenovirus protease. The active site is in the groove at the top where the "ball-and-stick" figures are located. The cofactor is the strand in the center at the bottom. The DNA binding sites are identified with "plus (+)" signs.